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Seroepidemiological Study of Epstein-Barr Virus Infection in Bangladesh

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A seroepidemiological study was carried out on 502 sera to determine the prevalence of EBV infection in a group of Bangladeshi people (age range: 15 days–90 years). All sera were tested for IgG antibody to the EBV viral capsid antigen (VCA) by a commercially available enzyme linked immunosorbent assay (ELISA) and the negatvie sera were checked subsequently by indirect immunofluorescence (IF) methods. The prevalence of EBV infection in the study group was 81.27%. 42.37% of infants had antibodies to EBV by the age of 1 year. A significant rise in the percentage of seropositives between 0–1- and 1–2-year-old children was demonstrated, indicating a high rate of primary infection at these ages. The prevalence of IgG antibody to VCA was 87.93% in the 2–10 years age group and was sustained at over 85% thereafter. Higher ELISA values were more common both in the 0–2- and >25-year age groups, the latter being statistically significant ($P < 0.025$). Similar higher values were also observed in females as compared to males ($P = 0.05$). Eighteen out of 109 negative sera and two equivocal sera by ELISA were found to be positive by indirect IF, indicating a negative predictive value of 82% for ELISA. The concordance between the two methods was 97% with ELISA proving to be less sensitive than indirect IF. It is concluded that the prevalence of EBV infection in Bangladeshi population is similar to that observed in other developing countries and that ELISA can be used for seroepidemiological surveys; however, the sera negative by ELISA should be checked routinely by indirect IF.

KEY WORDS: Epstein-Barr virus, seroepidemiology, anti-VCA IgG antibody, immunofluorescence, ELISA.

INTRODUCTION

Epstein-Barr virus (EBV), a human herpes virus, has a worldwide distribution with ~90% of the adult population showing evidence of past infection [Henle and Henle, 1966]. Seroepidemiological surveys have demonstrated antibodies to EBV antigens in every population studied so far, including some remote isolated tribes in Alaska and the Amazon basin [Tischendorf et al., 1970; Black et al., 1974]. Significant differences however, exist in the prevalence rate, age-specific incidence of primary infection, and type of clinical manifestations. In developing countries, EBV infection occurs very early in life, shortly after the decline of maternal antibodies [Biggar et al., 1978], and most children have seroconverted by the age of 6 years. However, in developed countries there is a gradual increase in the prevalence of infection with increasing age and only 30–40% are seropositive by 6 years of age [Evans, 1982]. Of individuals whose primary infection is delayed until adolescence, 50% develop acute infectious mononucleosis (IM) [Niederman et al., 1970], a self-limited disease characterized by fever, pharyngitis, and lymphadenopathy. The diagnosis of IM is established by the presence of an atypical lymphocytosis and the typical antibody profile in serum [Henle et al., 1974]. This is characterized by the presence of IgM antibody to EBV viral capsid antigen (VCA) and rising titres of IgG antibodies to VCA and early antigens (EA). During this period, there is also a pronounced non-specific response to autoantigens and heterophile antibodies (HA) of IgM class, which agglutinate red cells from species other than humans and are found in 85% of patients. These antibodies form the basis of Paul-Bunnell test and the rapid slide agglutination diagnostic test for IM [Davidson et al., 1971]. In convalescence, IgM anti-VCA and IgG anti-EA levels decline, whereas IgG anti-Epstein-Barr nuclear antigen 1 (EBNA 1) antibodies only become detectable during this period. Following recovery from primary infection, IgG anti-VCA and IgG anti-EBNA antibodies persist in serum for life in immunocompetent individuals.

Little is known about the EBV infection in the Southeast Asian regions. This study was designed to assess

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the seroepidemiology of EBV infections in Bangladesh, a developing country, by looking at the antibodies to EBV VCA and to determine the age of primary infection in such a population. Antibodies to VCA are conventionally measured by the indirect immunofluorescence (IF) method. A common problem encountered with indirect IF is nonspecific immunofluorescence staining, usually due to the presence of autoantibodies [Klein et al., 1967]. Further problems can occur in determining the end points of titration, because reference sera are not available, nor is there any form of quality control to ensure standardisation of results. Standardisation is also difficult due to the variable numbers of antigen-producing cells in the positive cell lines used in the assay as well as to the subjective interpretation of results [Hotchin and Crawford, 1991]. Therefore, IF assays are time-consuming, particularly for large-scale seroepidemiological testing, and need expert personnel and expensive equipment, which may not always be available in developing countries. Recently, attempts have been made to simplify and improve EBV serodiagnosis by using ELISA. ELISAs have been developed for detecting antibodies to VCA complex using purified protein from EBV infected cell lines, synthetic peptides or recombinant antigens [Thiele et al., 1987]. Results indicated a high degree of correlation between ELISA and IF titres [Luka et al., 1984]. In one study, ELISA was found to be more sensitive than IF methods [Dolken et al., 1984]. In this study, the efficiency of commercially available ELISA as an alternative, more rapid tool for EBV seroepidemiological surveys was investigated.

MATERIALS AND METHODS

Sera

Serum was collected from 374 patients presenting with diarrhoeal diseases at ICDDR,B (International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh) and 128 patients attending a private pathology clinic in Chittagong (Bangladesh) for various routine clinical tests. Sera were kept at −20°C and transferred to the London School of Hygiene and Tropical Medicine frozen. Eighteen documented EBV positive and five EBV negative sera, obtained from the Department of Virology (Middlesex Hospital, London), were used as controls. In every experiment, known control IgG VCA positive serum (JAT) and EBV negative serum (WDA) were always included.

Detection of EBV Antibodies

The Clin-ELISA™ VCA IgG test system (INCSTAR Corp. Stillwater, MN, USA) was used for the detection of IgG antibody to EBV VCA in human serum by ELISA. This commercially available assay is based on purified protein components of the VCA complex and was carried out according to the manufacturer's instructions. Calibrators and control sera provided in the kit were run with each test run and quality control criteria were met. Results were read by an ELISA microplate reader (Dynatech, MR 5000). According to the manufacturer, a VCA IgG value of a 100 or less was considered a negative result, a value between 101 and 109 was equivocal, and a value of 110 or greater was considered a positive result.

Sera were also tested for IgG and IgM antibodies to EBV VCA and IgG antibodies to EA by standard methods of indirect immunofluorescence [Henle and Henle, 1966]. The anticomplement immunofluorescence (ACIF) method was used for detection of IgG antibodies to EBNA [Klein et al., 1976].

Detection of Heterophile Antibodies

Sera were screened for heterophile antibodies with the Monostat slide agglutination test (Colby, Chicago Heights, IL) following the manufacturer's instructions.

Stastical Analysis

The Chi-square test was used for the statistical analysis of the difference between two proportions. The McNemar's Chi-square test, based on the number of discordant pairs, was used to test the hypothesis that ClinELISA™ and indirect IF were equally good. A P value of <0.05 was taken as statistically significant.

RESULTS

Control Experiments

This set of experiments was desinged to assess the validity of Clin-ELISA™ as a mehtod for seroepidemiological surveys as compared to the conventional indirect IF method. The control sera with known EBV status were tested both by Clin-ELISA™ at 1 in 51 dilution (according to the manufacturer's instructions) and by indirect IF at 1 in 10 screening dilution. All five EBV negative sera were found to be negative by the ELISA. Three of the 18 positive sera were found to be negative by Clin-ELISA™ but gave positive results (>1 in 10) by indirect IF. So, in this initial control test, the two methods had concordant results in 87% of the sera. The value was considered acceptable and, therefore, since ELISA was a simpler and more rapid method than IF, we proceeded to screen all the test sera by Clin-ELISA™. All the sera negative by the ELISA were retested by indirect IF.

Comparison Between Clin-ELISA™ and Indirect IF

The level of immunity to EBV in the population tested, as indicated by IgG antibodies to VCA was 77.88% by Clin-ELISA™. Out of 502 sera, 391 were positive and 109 were negative for antibodies to EBV VCA, and two sera gave equivocal results. When checked by indirect IF for IgG to VCA, the two equivocal sera and 15 of the 109 negative sera were found to be positive in one in 10 dilution. The prevalence rate of IgG antibodies to VCA in the group tested was 81.27% as corrected after the use of indirect IF method (Fig. 1). Since all the borderline positives by Clin-ELISA™ gave positive results by indirect IF, we made the assumption that all the sera positive by ELISA would also be positive by indirect IF. Therefore, the concordance

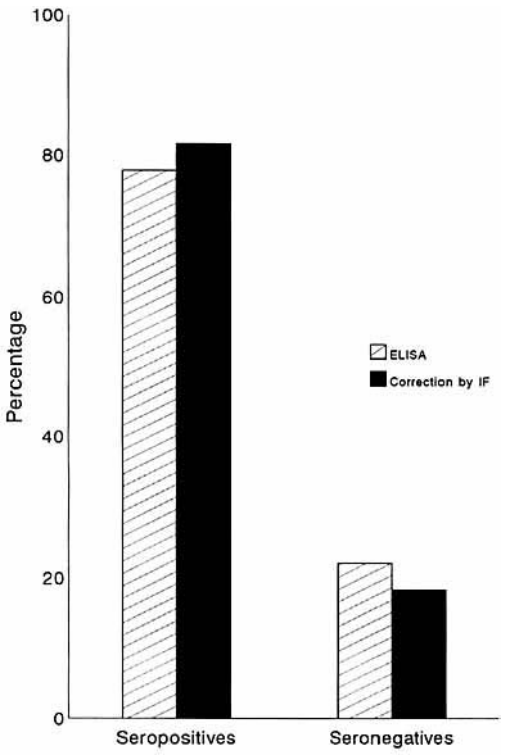

Fig. 1. Overall percentage of EBV seropositive and seronegative individuals in the study population determined by ELISA, before and after correction by indirect IF.

between the two methods was calculated to be 97%. Taking the indirect IF as the reference test, the sensitivity and negative prediction value of Clin-ELISA™ were found to be 96.3% and 82%, respectively (we also assumed that neither method gave false-positive results). The indirect IF proved to be significantly better than Clin-ELISA™ ($P < 0.003$) when analyzed by McNemar's Chi-square test. The specificity of Clin-ELISA™ was calculated to be 100%.

Age-Specific Prevalence of EBV Infections

Results were analyzed on the basis of age and sex of the population. Information on the age was not available for 50 patients, and they were excluded from further analysis. The demographic data on the study population is presented in Table I. In this study group, antibodies to EBV were acquired early in life; 42.37% had anti-VCA IgG by the age of 1 year, 40.62% within the first 6 months. There was a skewed increase at 1–2 years (92.3%) following which the percentage of seropositivity dropped to 87.93% in the 2–10-year age group, and thereafter remained stable without significant changes (Table II). The rise in seropositivity between the 0–1- and 1–2-year age groups was found to be statistically significant ($P = 0.003$). No significant difference was noted in the prevalence rate of anti-VCA IgG between males and females.

TABLE I. Demographic Data on the Study Population

Age groups (years)	No. male (%)	No. female (%)	Total no.
0–2	40 (56)	32 (44)	72
3–10	25 (43)	33 (57)	58
11–25	30 (55)	24 (45)	54
26–50	89 (63)	52 (37)	141
>50	60 (47)	67 (53)	127
Total	244 (54)	208 (46)	452

TABLE II. Age-Specific Prevalence of Epstein-Barr Virus IgG Anti-VCA Antibody

Age groups (years)	No. sera tested	No. positives (%)	No. negatives (%)
0–2	72	37 (51.4)	35 (48.6)
3–10	58	51 (87.9)	7 (12.1)
11–25	54	46 (85.2)	8 (14.8)
26–50	141	120 (85.1)	21 (14.9)
>50	127	115 (90.5)	12 (9.4)
Total	452	369 (81.6)	83 (18.4)

Prevalence According to ELISA Reactivity

Sera positive by Clin-ELISA™ were grouped as low (value range 110–400), high (value range 401–750), and very high (value <750) on the basis of their ELISA values (Fig. 2). Very high values were more common in the 0–2-year- and >25-year age group, the latter being statistically significant ($P < 0.025$). It was also observed that very high values were more common among females than among males in the total population tested with a borderline significance ($P = 0.05$).

Further EBV-Specific Serology

Since the age of seroconversion in the study population was 1–2 years, we tested for EBV specific antibodies to detect typical or atypical primary infection in this age range. Therefore, the very highly reactive sera (13) from the 0–2-year age group were studied further for IgM antibody to VCA, IgG antibodies to EA and EBNA and heterophile antibodies. Thirteen strongly positive sera from the > 25-year age group were also included as nonmatched controls (Table III); 85% fo the 26 highly reactive sera had antibody to EBNA 1 and 50% had antibody to EA. The number of IgG anti-EA positive sera was significantly higher in the >25-year age group ($P < 0.025$). Four sera (3 from the 0–2 year and 1 from the >25-year age group) were positive for HA. In one case (>25-year age group), the positive HA was accompanied by the presence of antibodies to EA and the absence of antibodies to EBNA 1, suggesting a recent primary infection.

DISCUSSION

EBV, like other herpes viruses, establishes a lifelong persistent infection in humans after the primary infection, which is evident by the detection of virus in the throat washing and in B lymphocytes [Golden et al., 1973] and is reflected by the persistence of serum anti-

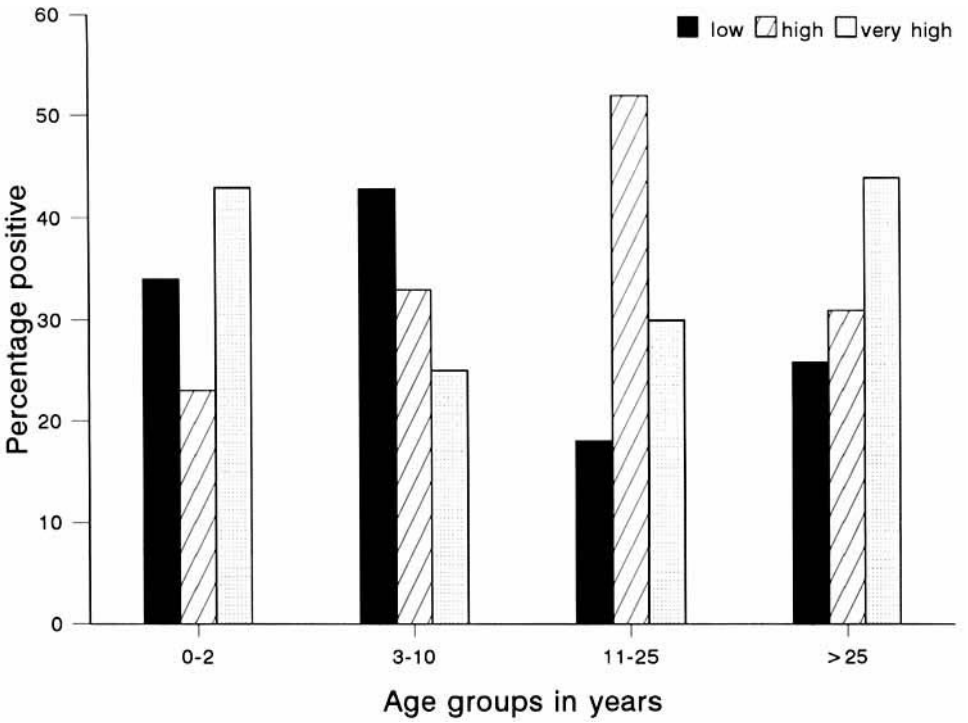

Fig. 2. Percentage of low, high, and very highly reactive sera in different age groups.

TABLE III. EBV-Specific Serology of Highly Reactive Sera

Age group (years)	Nos.	HA[a]	Anti-VCA IgM ab[b]	Anti-EA IgG ab	Anti-EBNA IgG ab
0–2	13	3	0	4	11
>25	13	1	0	9	11

[a]Heterophile antibodies.
[b]Antibodies.

bodies to EBV antigens. In this study of a group of Bangladeshi people, the overall prevalence of IgG antibody to EBV VCA was 82%; 43% of children < 1 year of age had antibody to EBV VCA as opposed to 88% of those in 2–10-year age group, with a significant peak of 92% in 1–2-year age group. The prevalence rate was sustained at >85% thereafter. This implies that the majority of children have acquired primary infection within the first 1–2 years of life. These findings are similar to those of other seroepidemiological studies in Ghana [Biggar et al., 1974], Mexico [Golubjatnikov et al., 1973], Uganda [Kafuko et al., 1971], and a low socioeconomic group in Houston, Texas [Porter et al., 1969]. Factors influencing the early acquisition of the virus may be the low level of hygiene, low socioeconomic status, and cultural practices [Lang et al., 1977]. As in other developing countries, early exposure to EBV infection probably accounts for the rarity of classical IM in young adult Bangladeshis [Tsega et al., 1987].

In contrast to the findings in developed countries where females were infected earlier than males [Heath et al., 1972], no significant difference in the male-female ratio at primary infection was noted. Nevertheless, a difference in sex was demonstrated, with seropositive females consistently having higher ELISA values than males in the group as a whole and in the 25–50- and >50-year age groups. This finding is comparable to that of other studies where higher anti-VCA antibody titres were found in females of various age groups [Sumaya et al., 1975]. Similarly, females have shown a greater antibody response to rubella vaccine, hepatitis B, poliovirus [Michaels et al., 1971], and cytomegalovirus [Luby et al., 1972].

The increased percentage of very high positive ELISA values in the 0–2-year age group may reflect the high rate of primary infection in children, and the second peak in the advanced age group may be associated with decline in immunoregulatory control facilitating the reactivation of the virus. Consequently, we tested 26 highly positive sera (13 from each of the 0–2-year and >25-year age group) for IgM to EBV VCA, HA, antibodies to EA, and EBNA 1. None appeared to have the profile classical to primary EBV infection. Similar to the above results, Hossain [1987] detected antibody

to EA only in the sera with high titre of anti-VCA antibodies in a group of Saudi people. The prevalence of EBV antibodies in the Bangladeshi study group conforms to the pattern seen in other nonindustrialised countries.

The sensitivity of the ELISA kit used in this study was lower than that of IF. Its specificity was 100%, a finding consistent with the manufacturer's performance data, which indicated a higher specificity (100%) than sensitivity (95%). Based on our results, this system can be used for rapid initial screening in seroepidemiological surveys, but the negative results should be checked. For that purpose, we used the indirect IF; however, alternative immunocytochemical methods, such as immunoperoxidase labelling of the second layer antibodies, may be more applicable in developing countries since expensive equipment is not required.

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